REMARKS/ARGUMENTS

The Pending Claims

Claims 1-12, 15, and 16 are pending and are directed to a method of producing pluripotent stem cells.

Summary of the Office Action

The Office rejects claims 1-12, 15, and 16 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement.

The Office rejects claims 1, 2, 4-6, and 15 as allegedly obvious under 35 U.S.C. § 103(a) over Hogan (U.S. Patent 5,690,926) and Creemers et al. (*Reproduction, 124:* 791-799 (2002)). The Office rejects claims 3, 7, and 16 under 35 U.S.C. § 103(a) over Hogan and Creemers et al. and further in view of one or more of the following: (i) Haneji et al. (*J. Endocrinol., 128(3)*: 383-388 (1991)), (ii) Wahab-Wahlgren et al. (*Mol. Cell. Endocrinol., 201*: 39-46 (2003)), (iii) Beumer et al. (*Cell Death and Differentiation, 5*: 669-677 (1998)), (iv) Kanatsu-Shinohara et al. (*Biol. Reprod., 69*: 612-616 (2003)), (v) Shinohara et al. (*Biol. Reprod., 66*: 1491-1497 (2002)), and (vi) Van Der Wee et al. (*J. Androl., 22(4)*: 696-704 (2001)).

Reconsideration of the rejections is hereby requested.

Discussion of the Enablement Rejection

The Office contends that the specification is not enabling for a method of producing pluripotent stem cells derived from the postnatal testis of any mammalian species. The Office cites to several references which allegedly detail the unpredictable nature of the field and the difference between pluripotent stem cells of mice and humans. In view of the alleged unpredictable nature of the field, the Office contends that the specification does not provide an adequate teaching for the full scope of the claims.

The specification describes the problems associated with the production of pluripotent stem cells from adult testis and specific methods to solve the problems. In particular, the specification identifies the problem to be solved in the production of pluripotent stem cells from adult testis cells as low frequencies of stem cells in adult testis. The specification provides specific guidance as to methods of solving the problem including the use of a

younger animal because younger animals have higher frequencies of stem cells (e.g., spermatogonial stem cells) contained in the testis (see, e.g., page 14, line 23, through page 15, line 5, and page 16, line 3-6). Accordingly, one of ordinary skill in the art would understand how to use the inventive methods with a reasonable expectation of success.

Post-filing references further evidence the success of the inventive methods described in the specification. In particular, Guan et al., *Nature, 440:* 1199-1203 (2006), and Guan et al., *Nature Protocol, 4*: 143-154 (2009) (copies of which are submitted herewith), disclose the generation of pluripotent stem cells from normal adult murine testis cells using the inventive methods. Conrad et al., *Nature, 456*: 344-351 (2008), and Kossack et al., *Stem Cells, 27*: 138-149 (2009) (copies of which are submitted herewith), disclose the generation of pluripotent stem cells from normal adult human testis cells using the inventive methods.

Furthermore, one of ordinary skill in the art at the time of filing the application would have been able to easily extrapolate the production conditions of human pluripotent stem cells from the production conditions of mouse pluripotent stem cells given the teachings in the prior art, such that no undue experimentation would be required to practice the inventive methods. For example, Hogan (U.S. Patent 5,690,926) demonstrates that pluripotent stem cells can be produced from human primordial germ cells under basically the same conditions as mouse embryonic germ cells (e.g., stem cell factor (SCF), basic fibroblast growth factor (bFGF), and leukemia inhibitory factor (LIF)) (see column 12, lines 14-60). In particular, Hogan describes that the methods of isolation of ES cells from murine embryos were repeated for isolation of ES cells from human embryos (see column 12, lines 17-19). Therefore, one of ordinary skill in the art would consider that the establishing conditions of embryonic germ cells in mouse and human basically are the same.

The Office cites to Turnpenney et al. (2006) as evidence that human EG cell derivation and culture include the use of additional additives than required for mouse EG cell derivation and culture. Among the additives described in Turnpenney et al. (2006), forskolin was considered to specifically act on human EG cells; however, forskolin also is reported to be effective for mouse EG cells (see Koshimizu et al., *Development, 122*: 1235-1242 (1996); a copy of which is enclosed herewith). While factors (e.g., forskolin) may improve the culture conditions of EG cells of mammals, Applicants note that the culture conditions for

human pluripotent stem cells do not differ much from the culture conditions for mouse pluripotent stem cells and that both require the use of GDNF as an essential factor (see, e.g., Hogan, Guan et al. (2006), Guan et al. (2009), Conrad et al., and Kossack et al.).

The Office contends that Aflatoonian et al. describes the difficulty of maintaining well-defined hEG cell lines through extended passage in culture, even though the initial generation of hEG cells is relatively simple. Applicants note that the pending claims are directed to a method of producing (i.e., generating) pluripotent stem cells from testis cells, and not to a method of maintaining human EG cells in culture over multiple passages. However, even if the maintenance of human EG cells is difficult, Applicants note that human EG cells have been established as described in Turnpenney et al., *Stem Cells, 21*: 598-609 (2003) (a copy of which is submitted herewith).

For the above-described reasons, the specification provides adequate enablement for the inventive methods, such that one of ordinary skill in the art at the time the application was filed would understand how to use the inventive methods with a reasonable expectation of success. Therefore, Applicants request that the enablement rejection be withdrawn.

Discussion of the Obviousness Rejections

The Office contends that it would have been obvious to one of ordinary skill in the art to arrive at the inventive methods based on the disclosures of the cited references. The obviousness rejections are traversed for the following reasons.

For subject matter defined by a claim to be considered obvious, the Office must demonstrate that the differences between the claimed subject matter and the prior art "are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." 35 U.S.C. § 103(a); see also *Graham v. John Deere Co.*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966). The ultimate determination of whether an invention is or is not obvious is based on certain factual inquiries including: (1) the scope and content of the prior art, (2) the level of ordinary skill in the prior art, (3) the differences between the claimed invention and the prior art, and (4) objective evidence of nonobviousness. *Graham*, 383 U.S. at 17-18, 148 U.S.P.Q. at 467.

Consideration of the aforementioned *Graham* factors here indicates that the present invention, as defined by the pending claims, is unobvious in view of the cited references.

As regards the scope and content of the prior art, the Office contends that Hogan discloses a method of making mammalian pluripotent ES cells by culturing postnatal mammalian testis in a composition comprising bFGF and LIF. The Office also contends that Hogan teaches isolating the ES cells from postnatal mammalian testis. The Office acknowledges that Hogan does not teach the use of GDNF; however, the Office contends that Creemers et al. teaches culturing spermatogonial cells in a medium containing GDNF, LIF, and bFGF. The Office contends that it would have been obvious for one of ordinary skill in the art to add GDNF to the culture system of Hogan because Creemers et al. suggests that optimization of the culture medium could improve viability or proliferation of spermatogonia. The Office relies on the remaining cited references to provide the features of the remaining dependent claims.

For purposes of the analysis here, and for the sake of argument, the level of ordinary skill can be considered to be relatively high, such that a person of ordinary skill in the art would have an advanced degree and/or several years of experience in the relevant field.

The present invention, as defined by the pending claims, is directed to a method of producing pluripotent stem cells, which comprises (a) culturing testis cells using a medium containing GDNF or an equivalent thereto, wherein the testis cells contain spermatogonial stem cells, and wherein the testis cells are derived from a postnatal mammal, and (b) isolating pluripotent stem cells from the cultured testis cells.

The method described in Hogan is substantially the same as the method of generating EG cells from primordial germ cells disclosed in Matsui et al., *Cell, 70(5)*: 841-847 (1992), which was previously cited by the Office. Brigid L. M. Hogan is listed as the inventor of Hogan and a co-author of Matsui et al. The Office cites to the method for the isolation of embryonic stem cell lines from postnatal mammalian testis set forth in the Example of Hogan at column 12, line 61, through column 13, line 13. Applicants note that this portion of the Example is written in present tense and provides no evidence that the method was actually performed.

At the time the application was filed, one of ordinary skill in the art believed that it was impossible to establish pluripotent stem cells from *postnatal* cells. As an example, Labosky et al. (*Development, 12*: 3197-3204 (1994)), which is a later-published reference from the same laboratory as the inventor of Hogan, reports that while pluripotent cell lines can be established from *primordial* germ cells of 8 days post coitum (p.c.) embryos and 12.5 days p.c. genital ridges, germ cells from the gonads of 15.5 days p.c. embryos and newborn mice (i.e., *postnatal* germ cells) did *not* give rise to embryonic germ cell lines under the conditions disclosed in the prior art (see, e.g., page 3199, paragraph bridging columns 1 and 2). Applicants note that Labosky et al. references experiments described in Matsui et al. which are the substantially the same as those described in Hogan. Therefore, one of ordinary skill in the art would have concluded from a consideration of the prior art disclosures (including Labosky et al.) that Hogan does not teach that pluripotent stem cells can be established from *postnatal* cells, as required by the pending claims.

Furthermore, none of the remaining cited references provides any credible reason to combine the isolation method of Hogan with the culturing method of Creemers et al. In view of the teachings of the prior art as exemplified by Labosky et al., one of ordinary skill in the art would not have been motivated to isolate and expand a culture of pluripotent stem cells from postnatal testis cells, as required by the pending claims, nor would one of ordinary skill in the art have reasonably expected such an approach to be successful.

The inventors recognized that pluripotent stem cells could be established from postnatal mammals without destruction of embryos or genetic modification using the inventive method. The inventive method circumvents the ethical problem of destroying embryos in the production of pluripotent stem cells, which is surprising and unexpected in view of the methods disclosed in the prior art (see, e.g., Labosky et al.).

Considering all of the *Graham* factors together, it is clear that the present invention – as defined by the pending claims – would not have been obvious to one of ordinary skill in the art at the relevant time in view of the combined disclosures of the cited references. Accordingly, the obviousness rejection should be withdrawn.

Conclusion

Applicants respectfully submit that the patent application is in condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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